The influence of the lower beak on the interorbital septum-prenasal process complex in the chick embryo

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SUMMARY

The effect of removal of the lower beak on the development of the interorbital septum-prenasal process (ISPP) complex was studied in chick embryos. In normal development the angle between the ventral contour of the interorbital septum and the long axis of the prenasal process increases. At the same time the angle between the ventral contour of the interorbital septum and the basal plate increases.

After surgical removal of the prospective lower beak at stage 29, the position of the entire ISPP complex was altered in stage-38 embryos and the prenasal process showed elongation. In stage-38 embryos in which the prospective upper beak had been removed at stage 29, Meckel's cartilage was elongated. It is concluded that straightening of the angle between the ventral contour of the interorbital septum and the long axis of the prenasal process is not influenced by the lower beak, whereas the position of the entire ISPP complex and the size of the prenasal process are under the epigenetic influence of the lower beak. The position and size of Meckel's cartilage are under the epigenetic influence of the upper beak.

INTRODUCTION

The development of the chick chondrocranium has been the subject of many investigations. Sonies (1907) gave a comprehensive description, and his nomenclature is still in use today. Romanoff (1960) gave an excellent review. The most recent account of chick chondrocranial development is found in Simons (1975).

In the course of ontogeny the angle between the long axis of the prenasal process and the ventral margin of the interorbital septum and the angle between the ventral margin of the interorbital septum and the basal plate, both increase (Lang, 1952; illustrations by Bellairs, 1958). Externally, after stage 32 (normal series of Hamburger & Hamilton, 1951) the position of the upper beak relative to the head changes (as seen in illustrations by Duval, 1889; and by Keibel & Abraham, 1900; also measured by Wouterlood, 1975). A change in the position of the upper beak after earlier prospective lower beak extirpation was found for chick embryos by Wouterlood (1975, 1976a).

Interpreted in a mechanical sense (Wouterlood, 1976a), the upper and lower

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beaks exert pressure on each other, as a result of which the size, shape and position achieved by the upper beak depends on the size, shape and position of the lower beak, and vice versa. However, as pointed out by Wouterlood (1976a), the gross appearance and position of the upper and lower beaks are independent of each other. These findings support van Limborgh’s (1970) concept of growth control in morphogenesis. According to this concept, morphogenesis of the skull is partially controlled by epigenetic and environmental factors in addition to intrinsic (genetic) factors. The finding of changes in the osseous elements of the upper beak in embryos without a lower beak raises the question of the extent to which the properties of the cartilaginous supportive structures in the upper beak, i.e. the prenasal process, the nasal capsule, and the interorbital septum, are dependent on the presence of the lower beak. The presence, size, shape, position, internal structure and biochemical composition of a given structure have been called its ‘properties’ (Dullemeijer, 1974). The size, shape, and position of the eye are known to act as epigenetic factors in the development of the interorbital septum. Unilateral microphthalmia (Coulombre & Crelin, 1958; Bellairs, 1958; Tonneyck-Müller, 1971/72; Wouterlood, 1977), and bilateral microphthalmia (Silver, 1962; Tonneyck-Müller, 1974, 1976), which were induced by eye extirpation or damage, led to alterations in the size and/or shape and/or position of the interorbital septum-prenasal process (ISPP) complex. Properties of the mandible which may act as epigenetic factors in the development of this complex have not been taken into account in the past. Wouterlood (1976a) showed an influence of the mandible on the elements of the upper beak. Thus, the mandible may influence the prenasal process and thus the nasal capsule and interorbital septum as well. However, in experiments in which eyes or parts of eyes are destroyed, damage to the interorbital septum is not excluded. Since extirpation of the prospective lower beak does not affect the eye (Wouterlood, 1976a), any damage to the interorbital septum via the eye is excluded in such experiments.

A quantitative description of the development of the ISPP complex is not available in the literature. We have therefore described quantitatively some developmental stages up to and including the stage in which the results of extirpation of the prospective lower beak were investigated. The impact on Meckel’s cartilage of removal of the prospective upper beak (reciprocal to lower beak removal) was analysed as well.

MATERIALS AND METHODS

White Leghorn eggs obtained from a commercial breeder were placed with the blunt end up in trays in a forced-draught incubator (temperature 38 °C; relative humidity 60–65 %). The embryos were staged according to Hamburger & Hamilton (1951) except in the second half of the incubation period, for which their method was adapted according to Wouterlood (1976b). Embryos for the
Fig. 1. Representative embryo of stage 38 according to Hamburger & Hamilton (1951). (A) Sham-operated embryo (CS); (B) embryo without a lower beak (CLB); (C) embryo without an upper beak (CUB). The bar represents 5.0 mm.
description of normal development were taken from untreated eggs. Six developmental stages were described (12 embryos per stage), i.e. 32, 34, 35, 36, 37, and 38.

Extirpation of the prospective lower or upper beak was performed on embryos of stage 29. The blunt end of the egg was gently washed twice with 70 % alcohol, after which a window (about 4 cm²) was made in the shell with forceps and the serosa and amnion were cut. With a pair of fine, hooked tungsten needles either the prospective lower beak distal to a transverse cut made at the buccal corner was removed (embryos without a lower beak, indicated as CLB) or the prospective upper beak distal to a transverse cut made at the nares (embryos without an upper beak, indicated as CUB). Because of the absence of myeloblasts in the extirpated areas, the formation of the jaw musculature was not affected.

The controls were sham-operated embryos (indicated as CS). The results were analysed in stage-38 embryos (30 embryos per group); survival percentages: CLB = 80, CUB = 83 and CS = 76. A photograph of a representative embryo of each group is given in Fig. 1. After 2 weeks of fixation in 70 % alcohol, the body weight was determined. For the various groups the mean cube root of the body weight was: stage 32, 0.93 ± 0.02; stage 34, 1.08 ± 0.03; stage 35, 1.24 ± 0.03; stage 36, 1.43 ± 0.03; stage 37, 1.47 ± 0.03; stage 38, 1.61 ± 0.06; CS, 1.61 ± 0.05; CLB, 1.61 ± 0.04; CUB, 1.61 ± 0.06. Next, the heads of all embryos were stained with Alcian blue according to Simons & van Horn (1970/71) for cartilage. After being cleared in glycerin, the heads were photographed in the lateral view. In the photographs (Fig. 2) the tip of the prenasal process (Pp), the rostral and caudal tips of Meckel's cartilage (rCM and cCM), the caudal tip of the parieto-tectal process (Ptc), the dorsal tip of the supraoccipital (So) and the ventro-rostral spine of the exoccipital (SpEx) were marked with India ink. In the experimental group the line between So and SpEx served both as a reference line and as the X axis of an orthogonal co-ordinate grid (SpEx being the origin). The photographs were enlarged such that the length of the reference line was 40.0 mm. On the sides of the triangle Pp-Ptc-SpEx, perpendicular lines were drawn dividing these sides or sections of them into equal parts. Ink marks were used to indicate the points at which the perpendicular lines intersected the contours of the interorbital septum or the prenasal process, and also some additional characteristic structures. For Meckel's cartilage, the procedure was as follows: the line between the rostral and caudal tips (rCM and cCM) was divided into four equal parts by perpendicular lines, and the points where these lines intersected the axis of Meckel's cartilage were marked with ink. The intersection of the axis of the retroarticular process (prrA), the axis of the lateral articular process (p1A), and the intersection of the axis of the lateral articular process and the anterior margin of this process, were also marked.

The mean co-ordinate values of the marked points were plotted for each
Fig. 2. Reduction of a photograph to a standardized series of marks on the contours of the ISPP complex and on Meckel's cartilage. For this purpose, an orthogonal coordinate grid is drawn and the co-ordinates of the marks are mapped. The result is illustrated by the lower drawing. For a given developmental or experimental group, the co-ordinates of corresponding marks are then averaged and a ‘paper embryo’ constructed. $X$ and $Y$ are axis of the grid; each photograph was enlarged such that the length of the reference line (Ref.) was 40-0 mm.
group, and an average ‘paper embryo’ was then constructed. The embryos of the developmental series were photographed also with an external calibration bar.

The data from the photographs were processed automatically (developmental series) or manually (experimental series). Comparison of the paper embryos of the experimental group was based on superposition of the co-ordinate grids.

RESULTS

1. Development of the ISPP complex

Comparison of the ISPP complexes of the developmental stages (Fig. 3) reveals the following:

1.1. The angle between the reference line and the line drawn through the tip of the prenasal process (Pp) and the caudal point of the interorbital septum (D) increases gradually in the course of development (19°, 22°, 27°, 36°, 36-5°, and 41° for stages 32, 34, 35, 36, 37, and 38, respectively). Thus, the angle between the basal plate and the ventral contour of the interorbital septum increases.

1.2. The ratio between the distance from the tip of the prenasal process to the caudal point of the interorbital septum (Pp-D) and the distance from the tip of the prenasal process to the caudal tip of the parietal process (Pp-Ptc) increases rapidly between stages 32 and 36 (0.84, 1.03, 1.05, and 1.10 for the respective stages), but decreases slightly between stages 36 and 38 (1.10, 1.08,
Fig. 4. Comparison of the ventral contours of the ISPP complexes of the developmental stages on a relative basis (stages 32–37 enlarged to the size of stage 38). R = rostral; C = caudal.

and 1·06 for stages 36, 37, and 38). Thus, the ventral side of the ISPP complex grows faster than the dorsal side between stages 32 and 36, but the reverse occurs between stages 36 and 38, albeit to a lesser degree.

1.3. The ratio between the distance from the ventral tip of the planum antorbitale to the tip of the prenasal process (Pa-Pp) and the distance from the ventral tip of the planum antorbitale to the caudal point of the interorbital septum (Pa-D) increases rapidly up to stage 36 and more slowly in the succeeding stages (1·05, 1·34, 1·45, 1·50, 1·50, and 1·56 for stages 32 to 38). This indicates a stronger growth in the rostral part of the ISPP complex relative to the caudal part.

1.4. The angle between the ventral contour of the prenasal process and the long axis of Meckel's cartilage decreases between stages 32 and 36 and then remains constant.

1.5. The tip of the prenasal process becomes distinctly curved in the ventral direction in stages 36, 37, and 38.

Comparison of the overall appearance of the ISPP complex at various developmental stages (Fig. 4) reveals that the ventral contour of the ISPP complex forms an arch which straightens between stages 32 and 36; at stage 37, however, this straightening is interrupted, and at stage 38 the curvature of the ventral contour is similar again to that of stage 35.

2. Experimental series

The paper embryos of each group are shown in Fig. 5. Unless otherwise stated, the elements were compared by superposition of the co-ordinate grids.
2.1. Comparison of embryos without a lower beak (CLB) and sham-operated embryos (CS)

In CLB embryos the ventral and dorsal contours of the ISPP complex show rotation in the ventral direction. The centre of this rotation is situated in the connexion between the septum and the basal plate, near the caudal point of the interorbital septum (point D). The posterior part of the caudal contour of the interorbital septum has, however, the same position and shape in both groups.

In CBL embryos the remnant of Meckel’s cartilage shows translation in the
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rostral direction and rotation in the dorsal direction. Superposition of the ventral and dorsal contours of the ISPP complex showed complete conformity of the shape of these contours in both groups. In this mode of superposition the caudal contour shows a more posterior position in the CLB embryos.

The prenasal process, measured from the tip to the rostral margin of the nasal capsule, is larger in CLB embryos. The length of the pariotectal cartilage, measured from the rostral margin of the nasal capsule to the caudal tip of the pariotectal process, is the same in both groups. The position of the prenasal process and the nasal capsule relative to the interorbital septum is exactly the same in both groups.

2.2. Comparison of embryos without an upper beak (CUB) and sham-operated embryos (CS)

The CUB embryos lack the prenasal process and part of the nasal capsule. The interorbital septum shows rotation in the dorsal direction, the centre of which is located in the connexion between the interorbital septum and the basal plate (point D). The anterior part of the caudal margin of the interorbital septum has a more caudal and dorsal position in CUB embryos. Comparison of the interorbital septum of CUB and CS embryos with superposition of the ventral contours of the septum reveals good conformity of the shape of the ventral contours of the septum in both groups. The nasal capsule is rudimentary in CUB embryos, where the pariotectal process is shorter.

Meckel's cartilage shows elongation, and in CUB embryos the tip has a more dorsal and rostral position. In these embryos the retroarticular process shows retraction and a curvature of Meckel's cartilage in the dorsal direction is easily distinguished.

DISCUSSION

The developmental series shows that in the stages under study the greatest changes in the size and shape of the interorbital septum take place between stages 32 and 35. Before stage 32 the main events are the dorsal outgrowth of the interorbital septum from the trabecula communis and the fusion of the interorbital septum with the anterior and posterior orbital cartilages (Sonies, 1907; Romanoff, 1960). On the eighth day of incubation (Romanoff, 1960; this stage is comparable with stage 30 of the Hamburger & Hamilton series) the angle between the trabecula communis and the prenasal process is the same as at stage 32. Thus, straightening of the ventral contour of the interorbital septum-prenasal process complex occurs between stages 32 and 35.

Before stage 32, the upper and lower beaks grow spatially isolated from each other. From stage 32 on, the tip of the upper beak touches the tip of the lower beak (Hamburger & Hamilton, 1951). From the 6th (Kuo, 1932) or 6-5th (Hamburger, 1968) day of incubation, beak clapping can be observed in chick embryos.

On the basis of these facts taken together, the following working hypothesis
was developed for this study: the presence and the activity of the lower beak influences the shape of the ventral contour of the ISPP complex. The results of the experiments show a change in the position of the entire ISPP complex as well as elongation of the prenasal process when the lower beak is absent. In the absence of the upper beak, the size and curvature of Meckel's cartilage change together with the position of the interorbital septum. Hence, we arrive at the conclusion that in the normal embryo the presence of an upper and a lower beak is obligatory to establish some but not all of the normal properties of the ISPP complex. Therefore, the upper and lower beaks can be added to the list of elements acting as epigenetic factors on the control of the development of the ISPP complex in terms of van Limborgh's (1970) concept. According to this concept, the gross formation of a cartilaginous ISPP complex must be regarded as a process controlled by intrinsic factors and the attainment of its final size, shape and position seen as a result of the intrinsically controlled growth plus epigenetic and environmental factors.

In the normal embryo the presence of the lower beak has major consequences for the position of the entire ISPP complex and for the size of the prenasal process, minor consequences for the shape of the prenasal process, and no consequences for the size or shape of the interorbital septum, including the shape of its ventral contour and that of the prenasal process. The mutual position of the interorbital septum, nasal capsule, and prenasal process remains the same in CLB and CS embryos. Therefore, the working hypothesis must be rejected. The correct conclusion to be drawn from this study is that the process leading to the increase of the angle between the long axis of the prenasal process and the ventral contour of the interorbital septum (Lang, 1952; in illustrations by Bellairs, 1958; and confirmed in this study) is not changed by the absence of the lower beak and hence should be considered to be controlled independently of the lower beak.

In a qualitative study, Silver (1962) observed a normal relationship between the interorbital septum, nasal capsule, and the prenasal process after grafting of isolated ISPP preparations. On the other hand, in illustrations by Tonneyck-Müller (1974, 1976) it can be seen that both the ventral contour of the interorbital septum and the angle between the interorbital septum and the prenasal process are aberrant in bilaterally microphthalmic chick embryos, which indicates the importance of the eyes as epigenetic influences on the interorbital septum (see also the Introduction above). An influence of the eye was indeed found in CLB embryos. Superposition of the co-ordinate grids of CLB and CS embryos revealed rotation of the dorsal and ventral contours of the ISPP complex in the former, whereas the position of the caudal contour was the same in both groups. This means that the eye exerts a stronger influence on the caudal part of the interorbital septum than do the prenasal process and the nasal capsule. However, the role of the nasal capsule has never been studied satisfactorily. It is clear that the problem of the extent to which the increase in the
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The angle between the prenasal process and the interorbital septum is an intrinsic process can only be solved by thorough analysis of all possible epigenetic factors with an influence on this process. The main conclusion drawn here is that the lower beak is not such a factor.

Elongation of Meckel's cartilage in CUB embryos was also seen in earlier experiments (Wouterlood, 1976a, 1977), and elongation of the lower beak was always associated with improper fit of the beaks (Wouterlood, 1977). The present results show elongation of the prenasal process in the absence of a lower beak. Together, these results suggest a reciprocal influence of the upper and lower beaks during morphogenesis. The data obtained by measuring external dimensions (Wouterlood, 1975) pointed in the same direction. The mechanism underlying the effect probably acts on the cellular level and possibly via the cartilage of the chondrocranium, which, in turn, serves as a mould for the bones of the upper beak and of the mandible. Many other possible mechanisms can be proposed, however.

The authors are indebted to Professor P. Dullemeijer and to Dr I. Tonneyck-Müller for their valuable criticism and advice, to Mr S. Zeilstra, who stained the specimens, and to Mr S. Speelman, who prepared the illustrations.

List of Abbreviations

D caudal point of the interorbital septum on its caudal contour.
cCM caudal tip of Meckel's cartilage
Pa caudo-ventral point of the planum antorbitale
p1A lateral articular process of the mandible
Pp tip of the prenasal process
prrA retroarticular process of the mandible
Ptc caudal tip of the parietectal process
rCM rostral tip of Meckel's cartilage
So dorso-caudal tip of the basal plate under the ossification site of the supraoccipital bone. Referred to as dorsal tip of the supraoccipital
SpEx ventro-rostral spine of the basal plate under the ossification site of the exoccipital. Referred to as ventro-rostral spine of exoccipital

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(Received 16 June 1978, revised 25 September 1978)